Effect of Recombinant Human Platelet-activating Factor-Acetylhydrolase on Allergen-induced Asthmatic Responses

NOREEN R. HENIG, MOIRA L. AITKEN, MARK C. LIU, ALBERT S. YU, and WILLIAM R. HENDERSON, Jr.

Department of Medicine, University of Washington, Seattle, Washington; Department of Medicine, Johns Hopkins University, Baltimore, Maryland; and ICOS Corporation, Bothell, Washington

Platelet-activating factor (PAF) is a potent lipid mediator associated with key features of asthma such as airway constriction, eosinophil infiltration, edema, and mucus accumulation. Regulation of PAF occurs primarily through degradation to biologically inactive lyso-PAF by cellular and secreted PAF-acetylhydrolase (PAF-AH). We evaluated the effect of human recombinant PAF-AH (rPAF-AH) on the dual phase asthmatic response in atopic subjects with mild asthma. Effects on induced sputum cell counts and differentials, eosinophilic cationic protein (ECP), and tryptase were evaluated. Enrolled subjects demonstrated a positive skin test and a dual asthmatic response to allergen inhalation challenge. Fourteen subjects received rPAF-AH (1 mg/kg) or placebo intravenously in a randomized, double blind, placebo-controlled, two-period crossover study. Treatment with rPAF-AH did not significantly reduce either the early- or late-asthmatic response. Sputum eosinophil cell counts were not affected by treatment, but there was a trend toward a reduction in sputum neutrophils. No significant change in sputum ECP and tryptase was observed between rPAF-AH and placebo. Thus, at the dose studied, the unique anti-PAF agent rPAF-AH demonstrated no significant effect on the allergen-induced dual-phase asthmatic response.

Platelet-activating factor (PAF), a family of lipid mediators, may be important in the pathogenesis of asthma. Synthesis of PAF, 1-*O*-alkyl-2-acetyl-*sn*-glyceryl-3-phosphorylcholine, occurs in inflammatory cells by the action of a unique phospholipase A_2 and acetyltransferase on membrane phospholipids (1). Elevated PAF plasma levels have been found in patients with asthma with active symptoms (2) and after allergen challenge (3). The number of PAF receptors is significantly increased in the lung tissue of patients with asthma (4). In humans, aerosolized inhaled PAF induces bronchoconstriction, an increase in methacholine-induced airway hyperreactivity (AHR), and release of cysteinyl leukotrienes (1). Inhaled PAF also causes gas exchange abnormalities by increasing blood flow through poorly ventilated regions (5). Regulation of the proinflammatory effects of PAF is a balance between synthesis and degradation.

Degradation of extracellular PAF occurs when platelet-activating factor-acetylhydrolase (PAF-AH), a 43-kD serum protein encoded by genes on chromosome 6, catalyzes the conversion of active PAF to inactive lyso-PAF (6). Recently, homozygosity for a genetic missense mutation (V279F) in the PAF-AH gene that results in enzymatically inactive PAF-AH has been linked with severe asthma in the Japanese population (7). rPAF-AH has substrate specificity and enzymatic activity comparable to native PAF-AH and antiinflammatory effects on PAF-induced inflammation in both *in vitro* and *in vivo* studies (6). In a murine model of asthma, rPAF-AH inhibits allergen-induced AHR to methacholine, pulmonary eosinophilia, and mucus accumulation in the airways (8).

In this Phase IIa study, we examined whether enhanced degradation of PAF by increasing PAF-AH levels would diminish the late asthmatic response (LAR) to allergen challenge in subjects with mild asthma. The study aims were to (*1*) evaluate the safety of rPAF-AH administered intravenously as a single dose to subjects with stable asthma, and (*2*) determine the effect of rPAF-AH on the allergen-induced LAR.

METHODS

Subjects

All subjects gave written informed consent to this study that was approved by the Human Subjects Committees of the University of Washington and Johns Hopkins University. Subjects had a history of wheezing or chest tightness, and asthma was previously diagnosed by a physician. Subjects were clinically stable in the 2 mo prior to the study, had no other major medical conditions, controlled their symptoms with short-acting β-agonists alone, and were atopic. Females of childbearing age not using an acceptable form of contraception, tobacco smokers, and subjects with abnormalities in baseline serum hematologic or chemistry studies or electrocardiogram (ECG) were excluded. Inhaled sympathomimetics and caffeinated beverages were withheld for at least 8 h prior to each study visit. Thirty-four patients with asthma initiated screening, 16 of whom met entry criteria. Fourteen subjects with mild atopic asthma participated in and completed this study. A fifteenth subject entered the study, completed baseline screening and one of the two treatment periods, and then exited for reasons unrelated to the study. This subject was not included in the final analysis. A sixteenth subject met all eligibility criteria but moved away from the study site area prior to enrollment.

Study Design

This was a randomized, double-blind, placebo-controlled, two-period crossover study evaluating rPAF-AH at a dose of 1 mg/kg or placebo. The rPAF-AH was administered intravenously with a target plasma PAF-AH level of > 10 μg/ml based on the antiinflammatory effect found in animal studies. Placebo was the buffer solution for rPAF-AH delivered intravenously in an equivalent volume. Safety, plasma PAF-AH levels, and response to inhaled allergen challenge were evaluated.

Study Protocol

Screening. Screening occurred on three separate days over 3 wk. At the first visit, all subjects underwent an initial visit to demonstrate a normal physical examination, ECG, and laboratory tests including hematology, clinical chemistry, urinalysis, and a negative pregnancy test in women of childbearing potential. Allergen skin prick testing was performed to demonstrate atopy to the allergens (cat pelt, house dust mite, Timothy grass, birch, and ragweed) used for the allergen inhala-

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Dr. Henig's present address is Department of Medicine, Stanford University, Stanford, California.

Correspondence and requests for reprints should be addressed to William R. Henderson, Jr., M.D., Department of Medicine, Box 356523, 1959 NE Pacific St., University of Washington, Seattle, WA 98195-6523. E-mail: joangb@u.washington.edu

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tion challenge. The allergen that caused the largest wheal or the allergen that produced a positive skin prick test giving the subject prolonged chest tightness by history was selected for inhalation challenges. Increasing dilutions of this allergen were then applied, and the lowest dilution with a positive skin prick test was used to predict the allergen dose for inhalation challenge. Methacholine (0.625–8 mg/ml) challenge was performed according to American Thoracic Society (ATS) guidelines (9). Subjects who did not exhibit a drop in $FEV_1 \geq 20\%$ from the saline control with 8 mg/ml of methacholine were not included in the study. On the second screen visit, subjects underwent a baseline allergen bronchoprovocation challenge to demonstrate a dual response to asthma. To undergo allergen challenge, subjects' baseline FEV_1 was $\geq 70\%$ predicted. As previously reported by Cockcroft and coworkers (10), the predicted inhaled allergen provocative concentration (PC_{20}) was calculated as follows:

$$\log_{10}(\text{allergen } PC_{20}) = 0.68 \times \log_{10}(\text{methacholine concentration} \times \text{minimum allergen skin prick concentration})$$

Allergen challenge was performed according to the protocol of Fahy and coworkers (11). Serially increasing doubling doses of allergen were inhaled, and the cumulative dose resulting in a decrease in $FEV_1 \geq 20\%$ of the saline control defined the early asthmatic response (EAR). A LAR was defined as a drop in $FEV_1 \geq 15\%$ from the saline control that occurred more than 3 h after the challenge. Subjects who did not demonstrate a dual asthmatic response were not included in the study. Seven or more days after the baseline allergen challenge, a baseline hypertonic saline-induced sputum collection was performed using the protocol of Fahy and coworkers (11). Eosinophilic cationic protein (ECP) and tryptase in induced sputum samples were determined using sensitive radioimmunoassays (Pharmacia Diagnostics Inc., Fairfield, NJ). Subjects who met all screening inclusion criteria were randomized for participation in the study.

Treatment period 1. Vital signs, ECG, baseline FEV_1, and baseline laboratory exams were obtained 10 min prior to intravenous infusion of either rPAF-AH or placebo. Ten minutes after completion of the infusion, vital signs were repeated and allergen challenge was initiated. The initial dose of allergen was two doubling dilutions below the final dose of allergen they received in their baseline challenge. Spirometry was performed 10 min after the last inhalation. Two doubling concentrations of allergen up to their baseline dose were given until there was a decrease in $FEV_1 \geq 20\%$ or the baseline dose was administered. Spirometry was measured 20, 30, 45, and 60 min after the final dose of inhaled allergen then at 30 min intervals afterward up to 8 h. Plasma PAF-AH was measured at 0, 6, and 24 h, and 7 d after drug infusion. The concentration of PAF-AH was measured with a quantitative enzyme-linked immunosorbent assay that has a lower limit of detection of 0.2 g/ml. Twenty-four hours after drug infusion, subjects returned for laboratory examination and sputum induction. Subjects were also assessed 1 wk following drug infusion for any adverse events, plasma PAF-AH levels, and effect on serum chemistries or ECG.

Treatment period 2. The second treatment period occurred 2–4 wk after the first treatment period. The protocol was identical to treatment period 1.

Follow-up visit. Interim history of potential adverse effects, physical examination, and spirometry were performed 3 wk after the second drug treatment. Routine hematology, biochemistry, urinalysis, and pregnancy tests were rechecked.

Analysis of Data

All airway data are expressed as the mean and standard error of the mean (SEM) from the 14 subjects who completed both treatment periods. The extent of the asthmatic response was assessed as the maximal fall in FEV_1 from baseline expressed as a percentage change over time. The EAR was calculated from the area under the FEV_1/time curve between 0 and 90 min ($AUC_{0-90\ min}$), and the LAR was calculated from time 3 to 8 h ($AUC_{3-8\ h}$) after allergen challenge. Primary efficacy was assessed by evaluating the $AUC_{3-8\ h}$ following administration of rPAF-AH as compared with placebo. A standard ANOVA model for a two-way crossover design was utilized. Treatment, sequence, and treatment by sequence effects were examined. A p value < 0.05 was considered significant. Cell counts and differentials in the sputum samples were compared using a Student's two-tailed *t*-test. Subgroup analysis of the two centers did not show a difference between the sites for morning FEV_1, AUC for EAR or LAR, or sputum differential cell counts.

RESULTS

Adverse Events

Fourteen subjects (nine male/five female) completed the two-period crossover study. Adverse events are shown in Table 1. All adverse events were mild or moderate in intensity.

PAF-AH Serum Levels

Target levels of PAF-AH were defined as > 10 μg/ml based on animal models and Phase I human studies. All subjects had serum levels of PAF-AH > 10 μg/ml at 24 h after dosing (Figure 1).

TABLE 1

ADVERSE EVENTS OF rPAF-AH AND PLACEBO TREATMENTS

Adverse Events*	rPAF-AH	Placebo
Muscle soreness/achiness	1	2
Gastroenteritis	0	1
Nausea with vomiting	1	0
Headache	2	1
Upper respiratory tract infection	1	0
Chest achiness/tightness	2	1
Increased wheezing	1	1
Increased cough	0	1
Fever	1	9
Drowsiness/fatigue	3†	1
Nervousness	1	0
Insomnia	0	1

Definition of abbreviation: rPAF-AH = human platelet-activating factor-acetylhydrolase.

* Adverse events as reported by the subject. In all but one case, the causality of treatment administration and the adverse event was deemed either remote or unrelated by the investigator.

† In one of the three subjects, fatigue was attributed to administration of the study drug by the investigator.

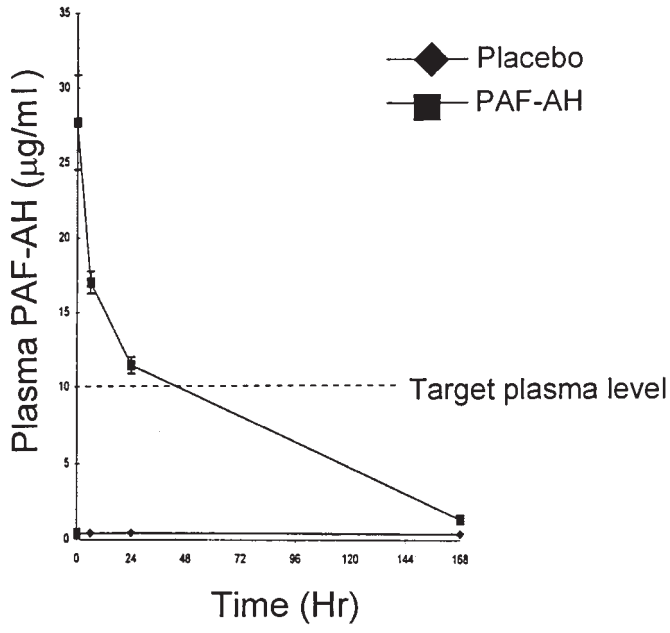

Figure 1. Blood levels of PAF-AH after intravenous administration of rPAF-AH. Plasma PAF-AH levels (μg/ml) were determined in subjects immediately after intravenous administration of rPAF-AH at a dose of 1 mg/kg or placebo, and 6 h, 24 h, and 1 wk later. The target level of > 10 μg/ml is shown by the *dashed line*.

One week after dosing, plasma PAF-AH levels were not significantly different from baseline levels or placebo (< 1 μg/ml).

Morning FEV_1

During the screening period, the mean morning FEV_1 was 3.19 ± 0.23 L (79.7% predicted). The mean morning FEV_1 was 3.06 ± 0.20 L before treatment with placebo and 3.06 ± 0.18 L before treatment with rPAF-AH. Twenty-four hours after drug treatment, the mean morning FEV_1 was 3.22 ± 0.21 L with placebo and 3.21 ± 0.22 L with rPAF-AH. There was no effect ($p > 0.05$) on morning FEV_1 measured 24 h after treatment with either rPAF-AH or placebo compared with pretreatment values.

Allergen-induced Early and Late Phase Asthmatic Responses

Seven subjects were challenged with cat pelt, two with dust mite, three with Timothy grass, and two with ragweed allergens. After the placebo treatment, 2 of 14 subjects had EAR only, 2 of 14 subjects had LAR only, 6 of 14 subjects had dual EAR and LAR responses, and 4 of 14 subjects had neither. After rPAF-AH treatment, 4 of 14 subjects had EAR only, 1 of 14 had LAR only, 7 of 14 had dual responses, and 2 of 14 had neither. The $AUC_{0–90\ min}$ was 1,904.7, 1,333.6, and 1,671.3 on the screening, placebo, and rPAF-AH allergen challenge days, respectively (Figure 2). The $AUC_{3–8\ h}$ was 4638.9, 3302.6, and 4880.4 on the screening, placebo, and rPAF-AH allergen challenge days, respectively. There was no difference between the rPAF-AH and baseline groups. There was a significant difference ($p < 0.05$) between the placebo and the other two groups for the LAR.

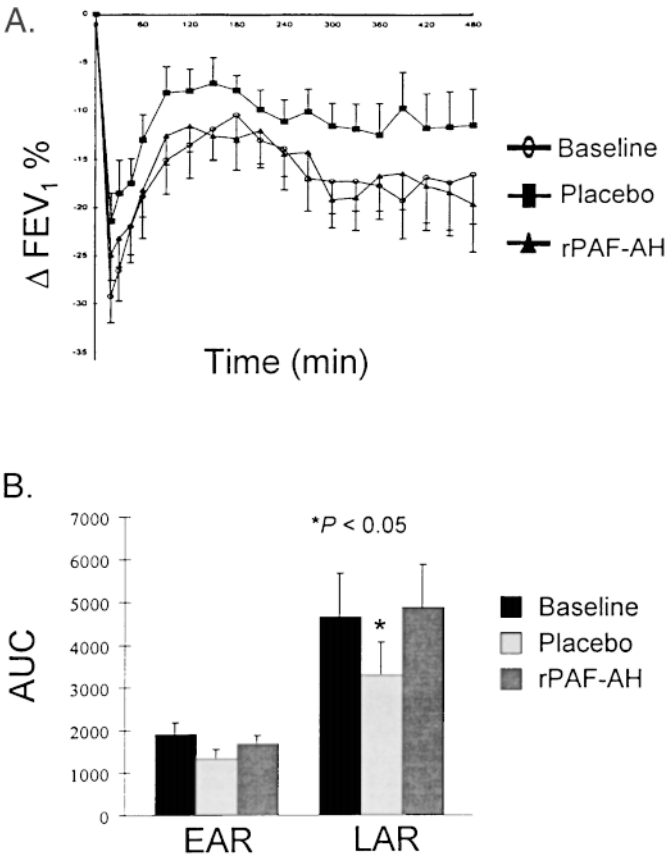

FIgure 2. Effect of rPAF-AH on the EAR and LAR following allergen challenge. In *A*, time points reflect a percentage change in FEV_1 percent (mean ± SEM) from the individual's postdiluent inhalation FEV_1. In *B*, the EAR and the LAR were calculated as the area under the curve between 0–90 min ($AUC_{0–90\ min}$) and 3–8 h ($AUC_{3–8\ h}$), respectively, after allergen challenge. A standard ANOVA model for a two-way crossover design was used to compare differences between groups. There was no significant difference between the three groups' EAR. A significant difference (*$p < 0.05$) was observed between the placebo LAR and other two groups. There was no significant difference between the rPAF-AH group and the baseline.

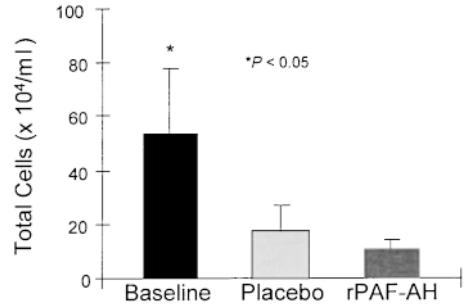

Figure 3. Effect of rPAF-AH on total cell counts in induced sputum. The number of total cells (mean ± SEM) was significantly greater at baseline than after either treatment (*$p < 0.05$ versus placebo or rPAF-AH). There was no significant difference between the rPAF-AH and placebo groups. Cell counts were compared using a Student's two-tailed *t*-test.

Induced Sputum Total Cell Counts

The total cell counts (mean ± SEM) in the induced sputum samples for baseline, rPAF-AH, and placebo were 50.2 ± 22.1, 10.3 ± 3.7, and 17.6 ± 9.0 cells × 10^4/ml, respectively (Figure 3). Whereas a significantly higher number of total cells was observed in the baseline group ($p < 0.05$), the total cell counts from the two treatment periods, when sputum induction followed allergen challenge, showed no significant difference.

Baseline induced sputum differential (mean percent ± SEM) were eosinophils 14.8 ± 3.2, neutrophils 43.7 ± 6.0, monocytes/macrophages 35.2 ± 5.4, lymphocytes 2.8 ± 0.8, and epithelial cells 3.7 ± 1.1. Induced sputum cell differentials 24 h after allergen challenge were as follows (mean percent ± SEM): neutrophils 47.6 ± 5.7 with placebo and 32.4 ± 5.6 with rPAF-AH; eosinophils 17.1 ± 3.6 with placebo and 23.9 ± 5.4 with rPAF-AH; monocytes/macrophages 31.5 ± 6.8 with placebo and 32.5 ± 5.4 with rPAF-AH; lymphocytes 1.4 ± 0.5 with placebo and 8.2 ± 4.2 with rPAF-AH; and epithelial cells 2.4 ± 0.7 with placebo and 2.9 ± 0.8 with rPAF-AH (Figure 4).

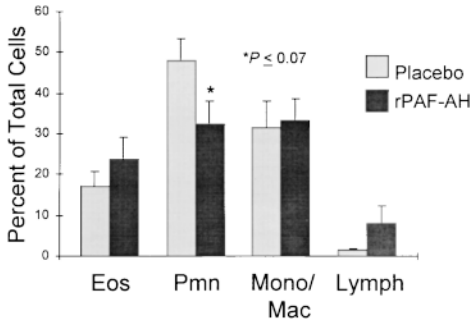

Figure 4. Effects of rPAF-AH on inflammatory cell count differentials in induced sputum 24 h after allergen challenge. A trend toward reduction in the percentage of neutrophils (Pmn) was found in the rPAF-AH-treated group (*$p \leq 0.07$). No significant differences were observed in eosinophil (Eos), monocyte/macrophage (Mono/Mac), or lymphocyte (Lymph) cell counts between the placebo and rPAF-AH groups. Cell differentials were compared using a Student's two-tailed *t*-test.

There was a trend toward a lower percentage of neutrophils in the sputum from the rPAF-AH group compared with the placebo group (*$p \leq 0.07$). There were no other significant differences between the two treatment groups.

Sputum Tryptase and ECP

Assays of sputum tryptase showed no significant difference among the baseline, placebo, or rPAF-AH treatment samples. Sputum ECP was 351.7 ± 149.3 ng/ml at baseline, 691.2 ± 374.7 ng/ml with placebo, and 660.3 ± 288.5 ng/ml with rPAF-AH treatment. There was no difference ($p > 0.05$) between the placebo and rPAF-AH ECP measurements.

DISCUSSION

This is the first study to examine the effects of rPAF-AH on allergen-induced airway responses in subjects with asthma. At a dose of 1 mg/kg, rPAF-AH was found to be safe in the 14 subjects studied. The one reported case of mild sleepiness associated with administration of the drug may be consistent with unpublished Phase I investigations in which transient drowsiness occurred at a dose of 3 mg/kg, but not 1 mg/kg. All investigators were aware of the Phase I findings. We found that rPAF-AH at a dose of 1 mg/kg intravenously did not attenuate either the EAR or LAR to bronchial allergen challenge in patients with mild asthma. However, rPAF-AH may affect the inflammatory cell differential as reflected in the reduction of neutrophils. Levels of tryptase and ECP in induced sputum obtained after allergen challenge were not affected.

Although several potent PAF receptor antagonists attenuate cellular and pulmonary responses to methacholine (12), PAF (13), and allergen challenge (14) in patients with mild asthma, most studies have found no effect of PAF receptor antagonists on the asthmatic response in humans (15–17). Our results are consistent with the studies that show no significant effect of PAF receptor antagonists on the LAR following allergen challenge. There are two possible explanations. One is that at the dose given, rPAF-AH did not effectively reduce PAF concentration in the lungs or PAF-induced bronchoconstriction. Alternatively, PAF may not play an important role in allergen-induced bronchoconstriction, and interfering with the effect of PAF may not be reflected in a change in spirometry.

PAF may still play a role in the airway cellular inflammation of patients with asthma. There is a recognized dissociation of AHR and cellular inflammation that may explain our results (18). This is supported by recent results that the potent PAF receptor antagonist SR 27417A attenuates inhaled PAF-induced effects on neutrophil levels (13). The trend toward a reduction of sputum neutrophils with rPAF-AH is an interesting, albeit unexpected result. A significant rise of sputum neutrophils is not usually associated with allergen bronchoprovocation after 24 h. Allergen challenge in dogs induces neutrophil proliferation in the bone marrow, leading to neutrophil trafficking through the circulation to lungs (19). Since PAF-AH acts in the extracellular compartment, adequate airway levels may not have been achieved to block the allergen-induced airway responses. However, the decrease in sputum neutrophils in the rPAF-AH-treated group suggests that there may have been an antagonistic effect of rPAF-AH on neutrophil trafficking into the lungs. Given this finding, future studies could target the therapeutic effect of rPAF-AH in patients with asthma in whom airway neutrophils are elevated such as those with acute asthma exacerbations (20) or corticosteroid-dependent severe asthma (21).

Why were the primary end points of this study, effect on LAR and sputum eosinophilia, negative, given the evidence for PAF in the pathogenesis of asthma? First, a different model other than the allergen bronchoprovocation model of asthma may have shown a larger effect. Second, although the study was powered appropriately for the primary endpoints, the variability of the methodology may have influenced our results. The unexpected results of the placebo group having a statistically significant reduction in LAR and the baseline sputum having significantly higher eosinophils reflect the variable nature of asthma as a disease and the dissociation of AHR and cellular infiltration of the airways (18). Although the crossover design was intended to minimize this variability, our results may have been affected by inter- and intrasubject issues and external factors such as recent exposures to allergens prior to study test days.

The dose and route of administration of rPAF-AH must also be considered in the interpretation of our results. The dose of 1 mg/kg may have been insufficient for therapeutic benefit. Our goal was to achieve plasma levels > 10 μg/ml over the 24 h of the treatment cycle. This level represents at least a 10-fold elevation over the normal range of 0.2–1.0 μg/ml (ICOS Corporation, unpublished data); although this level was antiinflammatory in animal studies, it may have been insufficient in humans. The discrepancy between the evidence for PAF as a proinflammatory mediator in patients with asthma and the negative Phase II trials of anti-PAF agents is intriguing. Although it has been suggested that there is no role for anti-PAF therapy for the treatment of asthma, it could be argued that the allergen bronchoprovocation model most commonly used in these trials does not accurately reflect the role of PAF (20). There is increasing evidence that asthma is a heterogeneous disease. PAF may play a greater role in the certain subsets of patients with asthma such as those individuals with more severe persistent asthma than examined in this study.

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